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=> s 2-hydroxyisoflavanone(w)synthase
L1          27 2-HYDROXYISOFLAVANONE(W) SYNTHASE

=> duplicate remove l1
DUPLICATE PREFERENCE IS 'AGRICOLA, BIOSIS, EMBASE, CAPLUS'
KEEP DUPLICATES FROM MORE THAN ONE FILE?  Y/(N):n
PROCESSING COMPLETED FOR L1
L2           14 DUPLICATE REMOVE L1 (13 DUPLICATES REMOVED)

=> d l2 1-14 ibib ab

L2   ANSWER 1 OF 14  CAPLUS  COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER:         2004:847623  CAPLUS
DOCUMENT NUMBER:          141:327639
TITLE:                    Cloning and characterization of 2-hydroxyisoflavanone
                          dehydratases from licorice and soybean and use in
                          isoflavonoid biosynthesis
INVENTOR(S):              Ayabe, Shinichi; Akashi, Tomoyoshi; Aoki, Toshio
PATENT ASSIGNEE(S):       Nihon University, Japan
SOURCE:                   PCT Int. Appl., 66 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 Japanese
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.           KIND   DATE        APPLICATION NO.          DATE
     ---------------      ----   --------    --------------------     --------
     WO 2004087909         A1    20041014    WO 2004-JP4214           20040325
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH,
             CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD,
             GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
             LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI,
             NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY,
             TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
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RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: JP 2003-92337 A 20030328

AB 2-Hydroxyisoflavanone dehydratase from licorice and soybean, encoding cDNAs, recombinant expression, and use in biosynthesis of isoflavonoids in combination with cytochrome P 450 ***2*** - ***hydroxyisoflavanone*** ***synthase*** , are disclosed. Transgenic plants transformed with those genes, esp., legumes, are claimed. CDNA encoding 2-hydroxyisoflavanone dehydratase was cloned from licorice (Glycyrrhiza echinata). It catalyzed conversion of 2,7-dihydroxy-4'-methoxyisoflavanone and 2,5,7-Trihydroxy-4'-methoxyisoflavanone into Formononetin and Biochanin A, resp. On the other hand, the 2-hydroxyisoflavanone dehydratase from soybean catalyzed conversion of 2,5,7-trihydroxyisoflavanone and 2,5,7,4'-tetrahydroxyisoflavanone into Daidzein and Genistein, resp. Thus, the enzyme from licorice is 2,7-dihydroxy-4'-methoxyisoflavanone 2,3-dehydratase (formononetin synthase) or HIDM (2-hydroxyisoflavanone dehydratase methoxy type) and the soybean enzyme is called 2,7,4'-trihydroxyisoflavanone 2,3-dehydratase (daidzein synthase) or HIDH (2-hydroxyisoflavanone dehydratase hydroxy type). Yeast cells overexpressing licorice ***2*** - ***hydroxyisoflavanone*** ***synthase*** (CYP93C2) and soybean HIDH was prepd. Both enzymes contained a carboxyl esterase motif, commonly present in lipases and esterases.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 2 OF 14 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN DUPLICATE 1

ACCESSION NUMBER: 2003:212507 BIOSIS

DOCUMENT NUMBER: PREV200300212507

TITLE: A cluster of genes encodes the two types of chalcone isomerase involved in the biosynthesis of general flavonoids and legume-specific 5-deoxy(iso)flavonoids in Lotus japonicus.

AUTHOR(S): Shimada, Norimoto; Aoki, Toshio; Sato, Shusei; Nakamura, Yasukazu; Tabata, Satoshi; Ayabe, Shin-ichi [Reprint Author]

CORPORATE SOURCE: Department of Applied Biological Sciences, Nihon University, Fujisawa, Kanagawa, 252-8510, Japan ayabe@brs.nihon-u.ac.jp

SOURCE: Plant Physiology (Rockville), (March 2003) Vol. 131, No. 3, pp. 941-951. print.
ISSN: 0032-0889 (ISSN print).

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 30 Apr 2003
Last Updated on STN: 30 Apr 2003

AB Leguminous plants produce 5-deoxyflavonoids and 5-deoxyisoflavonoids that play essential roles in legume-microbe interactions. Together with chalcone polyketide reductase and cytochrome P450 ***2*** - ***hydroxyisoflavanone*** ***synthase*** , the chalcone isomerase (CHI) of leguminous plants is fundamental in the construction of these ecophysiologically active flavonoids. Although CHIs of nonleguminous

plants isomerize only 6'-hydroxychalcone to 5-hydroxyflavanone (CHIs with this function are referred to as type I), leguminous CHIs convert both 6'-deoxychalcone and 6'-hydroxychalcone to 5-deoxyflavanone and 5-hydroxyflavanone, respectively (referred to as type II). In this study, we isolated multiple CHI cDNAs (cCHI1-cCHI3) from a model legume, Lotus japonicus. In contrast to previous observations, the amino acid sequence of CHI2 was highly homologous to nonleguminous CHIs, whereas CHI1 and CHI3 were the conventional leguminous type. Furthermore, genome sequence analysis revealed that four CHI genes (CHI1-3 and a putative gene, CHI4) form a tandem cluster within 15 kb. Biochemical analysis with recombinant CHIs expressed in Escherichia coli confirmed that CHI1 and CHI3 are type II CHIs and that CHI2 is a type I CHI. The occurrence of both types of CHIs is probably common in leguminous plants, and it was suggested that type II CHIs evolved from an ancestral CHI by gene duplication and began to produce 5-deoxy(iso)flavonoids along with the establishment of the Fabaceae.

L2 ANSWER 3 OF 14 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN DUPLICATE 2

ACCESSION NUMBER: 2004:267024 BIOSIS
DOCUMENT NUMBER: PREV200400269916
TITLE: Isoflavonoid biosynthesis and accumulation in developing soybean seeds.
AUTHOR(S): Dhaubhadel, Sangeeta; McGarvey, Brian D.; Williams, Ruthanne; Gijzen, Mark [Reprint Author]
CORPORATE SOURCE: Agr & Agri Food Canada, 1391 Sandford St, London, ON, N5V 4T3, Canada
gijzenm@agr.gc.ca
SOURCE: Plant Molecular Biology, (December 2003) Vol. 53, No. 6, pp. 733-743. print.
ISSN: 0167-4412 (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 26 May 2004
Last Updated on STN: 26 May 2004

AB Isoflavonoids are biologically active natural products that accumulate in soybean seeds during development. The amount of isoflavonoids present in soybean seed is variable, depending on genetic and environmental factors that are not fully understood. Experiments were conducted to determine whether isoflavonoids are synthesized within seed tissues during development, or made in other plant organs and transported to the seeds where they accumulate. An analysis of isoflavonoids by HPLC detected the compounds in all organs of soybean plant, but the amount of isoflavonoids present varied depending on the tissue and developmental stage. The greatest concentrations were found in mature seeds and leaves. The ***2*** - ***hydroxyisoflavanone*** ***synthase*** genes IFS1 and IFS2 were studied to determine their pattern of expression in different tissues and developmental stages. The highest level of expression of IFS1 was observed in the root and seed coat, while IFS2 was most highly expressed in embryos and pods, and in elicitor-treated or pathogen-challenged tissues. Incorporation of radiolabel into isoflavonoids was observed when developing embryos and other plant organs were fed with (14C) phenylalanine. Embryos excised from developing soybean seeds also accumulated isoflavonoids from a synthetic medium. A maternal effect on seed isoflavonoid content was noted in reciprocal crosses between soybean cultivars that differ in seed isoflavonoids. From these results, we propose that developing soybean embryos have an ability

to synthesize isoflavonoids de novo, but that transport from maternal tissues may in part contribute to the accumulation of these natural products in the seed.

L2 ANSWER 4 OF 14 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2004) on STN DUPLICATE 3

ACCESSION NUMBER: 2003:46173 AGRICOLA
DOCUMENT NUMBER: IND23332870
TITLE: Key amino acid residues required for aryl migration catalysed by the cytochrome P450 ***2*** - ***hydroxyisoflavanone*** ***synthase*** .
AUTHOR(S): Sawada, Y.; Kinoshita, K.; Akashi, T.; Aoki, T.; Ayabe, S.
AVAILABILITY: DNAL (QK710.P68)
SOURCE: The Plant journal : for cell and molecular biology, Sept 2002. Vol. 31, No. 5. p. 555-564
Publisher: Oxford : Blackwell Sciences Ltd.
ISSN: 0960-7412
NOTE: Includes references
PUB. COUNTRY: England; United Kingdom
DOCUMENT TYPE: Article
FILE SEGMENT: Non-U.S. Imprint other than FAO
LANGUAGE: English

AB Isoflavonoids are distributed predominantly in leguminous plants, and play pivotal roles in the interaction of host plants with biological environments. Isoflavones in the diet also have beneficial effects on human health as phytoestrogens. The isoflavonoid skeleton is constructed by the CYP93C subfamily of cytochrome P450s in plant cells. The reaction consists of hydroxylation of the flavanone molecule at C-2 and an intramolecular 1,2-aryl migration from C-2 to C-3 to yield 2-hydroxyisoflavanone. In this study, with the aid of alignment of amino acid sequences of CYP93 family P450s and a computer-generated putative stereo structure of the protein, candidates for key amino acid residues in CYP93C2 responsible for the unique aryl migration in ***2*** - ***hydroxyisoflavanone*** ***synthase*** reaction were identified. Microsomes of recombinant yeast cells expressing mutant proteins of CYP93C2 were prepared, and their catalytic activities tested. The reaction with the mutant in which Ser 310 in the centre of the I-helix was converted to Thr yielded increased formation of 3-hydroxyflavanone, a by-product of the ***2*** - ***hydroxyisoflavanone*** ***synthase*** reaction, in addition to the major isoflavonoid product. More dramatically, the mutant in which Lys 375 in the end of beta-sheet 1-4 was replaced with Thr produced only 3-hydroxyflavanone and did not yield the isoflavonoid any longer. The roles of these amino acid residues in the catalysis and evolution of isoflavonoid biosynthesis are discussed.

L2 ANSWER 5 OF 14 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 4

ACCESSION NUMBER: 2001045460 EMBASE
TITLE: Flavonoid 6-hydroxylase from soybean (Glycine max L.), a novel plant P-450 monooxygenase.
AUTHOR: Latunde-Dada A.O.; Cabello-Hurtado F.; Czittrich N.; Didierjean L.; Schopfer C.; Hertkorn N.; Werck-Reichhart D.; Ebel J.
CORPORATE SOURCE: J. Ebel, Botanisches Inst. der Universitat, Menzinger

Strasse 67, D-80638 Munchen, Germany.
j.ebel@botanik.biologie.uni-muenchen.de
SOURCE: Journal of Biological Chemistry, (19 Jan 2001) 276/3 (1688-1695).
Refs: 53
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Cytochrome P-450-dependent hydroxylases are typical enzymes for the modification of basic flavonoid skeletons. We show in this study that CYP71D9 cDNA, previously isolated from elicitor-induced soybean (Glycine max L.) cells, codes for a protein with a novel hydroxylase activity. When heterologously expressed in yeast, this protein bound various flavonoids with high affinity (1.6 to 52 .mu.M) and showed typical type I absorption spectra. These flavonoids were hydroxylated at position 6 of both resorcinol- and phloroglucinol-based A-rings. Flavonoid 6-hydroxylase (CYP71D9) catalyzed the conversion of flavanones more efficiently than flavones. Isoflavones were hardly hydroxylated. As soybean produces isoflavonoid constituents possessing 6,7-dihydroxy substitution patterns on ring A, the biosynthetic relationship of flavonoid 6-hydroxylase to isoflavonoid biosynthesis was investigated. Recombinant ***2*** - ***hydroxyisoflavanone*** ***synthase*** (CYP93C1v2) efficiently used 6,7,4'-trihydroxyflavanone as substrate. For its structural identification, the chemically labile reaction product was converted to 6,7,4'-trihydroxyisoflavone by acid treatment. The structures of the final reaction products for both enzymes were confirmed by NMR and mass spectrometry. Our results strongly support the conclusion that, in soybean, the 6-hydroxylation of the A-ring occurs before the 1,2-aryl migration of the flavonoid B-ring during isoflavanone formation. This is the first identification of a flavonoid 6-hydroxylase cDNA from any plant species.

L2 ANSWER 6 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:635036 CAPLUS
DOCUMENT NUMBER: 135:340707
TITLE: Properties and metabolic engineering of alfalfa phenylpropanoid pathway O-methyltransferases
AUTHOR(S): Dixon, Richard A.; Chen, Fang; He, Xian-Zhi; Noel, Joseph P.; Zubieta, Chloe
CORPORATE SOURCE: Plant Biology Division, Samuel Roberts Noble Foundation, Ardmore, OK, 73401, USA
SOURCE: Recent Advances in Phytochemistry (2001), 35(Regulation of Phytochemicals by Molecular Techniques), 131-154
CODEN: RAPHBE; ISSN: 0079-9920
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review, with refs. The authors describe the mol. characteristics of four alfalfa O-methyltransferases involved in the biosynthesis of flavonoids, isoflavonoids, and lignin. Genetic manipulation of the activities of three of these enzymes in transgenic alfalfa has been shown to have profound effects on pathway flux. Theses effects indicate useful strategies for crop improvement based on modification of

O-methyltransferase expression.
REFERENCE COUNT: 62 THERE ARE 62 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 7 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:553689 CAPLUS
DOCUMENT NUMBER: 133:146926
TITLE: Licorice ***2*** - ***hydroxyisoflavanone*** ***synthase*** cDNA, recombinant expression, and use in transgenic plants
INVENTOR(S): Ayabe, Shinichi; Aoki, Toshio; Akashi, Tomoyoshi
PATENT ASSIGNEE(S): Nihon University, Japan
SOURCE: PCT Int. Appl., 59 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000046356	A1	20000810	WO 2000-JP596	20000204
W: AU, CA, CN, JP, KR, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
PRIORITY APPLN. INFO.:			JP 1999-63745 A	19990204

AB Glycyrrhiza echinata ***2*** - ***Hydroxyisoflavanone*** ***synthase*** cDNA, primers, probes, antisense oligonucleotides, recombinant expression, and use in transgenic plants, are disclosed. Isoflavonoids are distributed predominantly in leguminous plants and play crit. roles in plant physiol. A cytochrome P 450, ***2*** - ***hydroxyisoflavanone*** ***synthase*** , is the key enzyme in their biosynthesis. In cultured licorice (Glycyrrhiza echinata L., Fabaceae) cells, the prodn. of both an isoflavonoid-derived phytoalexin (medicarpin) and a retrochalcone (echinatin) is rapidly induced upon elicitation. In this study, we obtained a full-length cDNA, CYP Ge-8 (CYP93C2), from the cDNA library of elicited G. echinata cells. When the flavanones liquiritigenin and naringenin were incubated with the recombinant yeast microsome expressing CYP93C2, major products emerged and were readily converted to the isoflavones daidzein and genistein by acid treatment. The chem. structures of the products from liquiritigenin (2-hydroxyisoflavanone and isoflavone) were confirmed by mass spectrometry. CYP93C2 was thus shown to encode ***2*** - ***hydroxyisoflavanone*** ***synthase*** , which catalyzes the hydroxylation assocd. with 1,2-aryl migration of flavanones. Northern-blot anal. revealed that transcripts of CYP93C2, in addn. to those of other P 450s involved in phenylpropanoid/flavonoid pathways, transiently accumulate upon elicitation.
REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 8 OF 14 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN DUPLICATE 5
ACCESSION NUMBER: 2001:80770 BIOSIS
DOCUMENT NUMBER: PREV200100080770

TITLE: New scheme of the biosynthesis of formononetin involving 2,7,4'-trihydroxyisoflavanone but not daidzein as the methyl acceptor.
AUTHOR(S): Akashi, Tomoyoshi; Sawada, Yuji; Aoki, Toshio; Ayabe, Shin-ichi [Reprint author]
CORPORATE SOURCE: Department of Applied Biological Sciences, Nihon University, Fujisawa, Kanagawa, 252-8510, Japan ayabe@brs.nihon-u.ac.jp
SOURCE: Bioscience Biotechnology and Biochemistry, (October, 2000) Vol. 64, No. 10, pp. 2276-2279. print.
ISSN: 0916-8451.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 14 Feb 2001
Last Updated on STN: 15 Feb 2002

AB Glycyrrhiza echinata cell-free extract produced isoformononetin by the 7-O-transmethylation of daidzein from S-adenosyl-L-methionine (SAM). When the yeast microsome expressing ***2*** - ***hydroxyisoflavanone*** ***synthase*** was mixed with the cell-free extract and incubated with liquiritigenin and SAM, formononetin emerged. Furthermore, the cell-free extract yielded formononetin on incubation with 2,7,4'-trihydroxyisoflavanone and SAM. We propose a novel pathway of formononetin biosynthesis involving 2,7,4'-trihydroxyisoflavanone as the methyl acceptor.

L2 ANSWER 9 OF 14 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN DUPLICATE 6
ACCESSION NUMBER: 2001:151075 BIOSIS
DOCUMENT NUMBER: PREV200100151075
TITLE: Induction of isoflavonoid pathway in the model legume Lotus japonicus: Molecular characterization of enzymes involved in phytoalexin biosynthesis.
AUTHOR(S): Shimada, Norimoto; Akashi, Tomoyoshi; Aoki, Toshio; Ayabe, Shin-ichi [Reprint author]
CORPORATE SOURCE: Department of Applied Biological Sciences, Nihon University, Fujisawa, Kanagawa, 252-8510, Japan ayabe@brs.nihon-u.ac.jp
SOURCE: Plant Science (Shannon), (December 7th, 2000) Vol. 160, No. 1, pp. 37-47. print.
CODEN: PLSCE4. ISSN: 0168-9452.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 28 Mar 2001
Last Updated on STN: 15 Feb 2002

AB Treatment of the seedlings of Lotus japonicus, a model legume for molecular genetic studies, with reduced glutathione (GSH) resulted in the accumulation of an isoflavan phytoalexin, vestitol. Using PCR strategies based on the conserved amino acid sequences, full length P450 cDNAs were obtained from GSH-treated seedling roots. When the clones, LjCYP-1 (CYP93C family) and LjCYP-2 (CYP81E family), were heterologously expressed in yeast, the proteins exhibited ***2*** - ***hydroxyisoflavanone*** ***synthase*** (IFS) and isoflavone 2'-hydroxylase (12'H) activities, respectively. The transcription levels of LjCYP-1, LjCYP-2 and isoflavone reductase, which are all involved in vestitol biosynthesis, coordinately increased upon elicitation. Genomic Southern blot analysis indicated that the IFS gene forms a small gene family and a single copy of the I2'H gene is present in the L. japonicus genome. Molecular biological aspects of

P450s involved in the isoflavonoid pathway and the genomic approach to flavonoid metabolism in this unique plant are discussed.

L2 ANSWER 10 OF 14 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2004) on STN DUPLICATE 7

ACCESSION NUMBER: 2000:60329 AGRICOLA
DOCUMENT NUMBER: IND22060334
TITLE: Cloning and functional expression of a cytochrome P450 cDNA encoding ***2*** - ***hydroxyisoflavanone*** ***synthase*** involved in biosynthesis of the isoflavonoid skeleton in licorice.
AUTHOR(S): Akashi, T.; Aoki, T.; Ayabe, S.
AVAILABILITY: DNAL (450 P692)
SOURCE: Plant physiology, Nov 1999. Vol. 121, No. 3. p. 821-828
Publisher: Rockville, MD : American Society of Plant Physiologists, 1926-
CODEN: PLPHAY; ISSN: 0032-0889
NOTE: Includes references
PUB. COUNTRY: Maryland; United States
DOCUMENT TYPE: Article; Conference
FILE SEGMENT: U.S. Imprints not USDA, Experiment or Extension
LANGUAGE: English

AB Isoflavonoids are distributed predominantly in leguminous plants and play critical roles in plant physiology. A cytochrome P450 (P450), ***2*** - ***hydroxyisoflavanone*** ***synthase*** , is the key enzyme in their biosynthesis. In cultured licorice (Glycyrrhiza echinata L., Fabaceae) cells, the production of both an isoflavonoid-derived phytoalexin (medicarpin) and a retrochalcone (echinatin) is rapidly induced upon elicitation. In this study, we obtained a full-length P450 cDNA, CYP Ge-8 (CYP93C2), from the cDNA library of elicited G. echinata cells. When the flavanones liquiritigenin and naringenin were incubated with the recombinant yeast microsome expressing CYP93C2, major products emerged and were readily converted to the isoflavones daidzein and genistein by acid treatment. The chemical structures of the products from liquiritigenin (2-hydroxyisoflavanone and isoflavone) were confirmed by mass spectrometry. CYP3C2 was thus shown to encode ***2*** - ***hydroxyisoflavanone*** ***synthase*** , which catalyzes the hydroxylation associated with 1,2-aryl migration of flavanones. Northern-blot analysis revealed that transcripts of CYP93C2, in addition to those of other P450s involved in phenylpropanoid/flavonoid pathways, transiently accumulate upon elicitation.

L2 ANSWER 11 OF 14 BIOSIS on STN DUPLICATE 8

ACCESSION NUMBER: 1999:362259 BIOSIS
DOCUMENT NUMBER: PREV199900362259
TITLE: Molecular characterization of the enzyme catalyzing the aryl migration reaction of isoflavonoid biosynthesis in soybean.
AUTHOR(S): Steele, Christopher L.; Gijzen, Mark; Qutob, Dinah; Dixon, Richard A. [Reprint author]
CORPORATE SOURCE: Plant Biology Division, Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK, 73401, USA

SOURCE: Archives of Biochemistry and Biophysics, (July 1, 1999) Vol. 367, No. 1, pp. 146-150. print. CODEN: ABBIA4. ISSN: 0003-9861.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 2 Sep 1999 Last Updated on STN: 2 Sep 1999

AB The first specific reaction in the biosynthesis of isoflavonoid compounds in plants is the 2-hydroxylation, coupled to aryl migration, of a flavanone. Using a functional genomics approach, we have characterized a cDNA encoding a ***2*** - ***hydroxyisoflavanone*** ***synthase*** from soybean (Glycine max). Microsomes isolated from insect cells expressing this cytochrome P450 from a baculovirus vector convert 4',7-dihydroxyflavanone (liquiritigenin) to 4',7-dihydroxyisoflavone (daidzein), most likely via 2,4',7-trihydroxyisoflavanone which spontaneously dehydrates to daidzein. The enzyme also converts naringenin (4',5,7-trihydroxyflavanone) to genistein, but at a lower rate. ***2*** - ***Hydroxyisoflavanone*** ***synthase*** transcripts are strongly induced in alfalfa cell suspensions in response to elicitation.

L2 ANSWER 12 OF 14 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2004) on STN DUPLICATE 9
ACCESSION NUMBER: 1999:21631 AGRICOLA
DOCUMENT NUMBER: IND21969338
TITLE: Purification of 2-hydroxyisoflavanone dehydratase from the cell cultures of Pueraria lobata.
AUTHOR(S): Hakamatsuka, T.; Mori, K.; Ishida, S.; Ebizuka, Y.; Sankawa, U.
CORPORATE SOURCE: Toyama Medical and Pharmaceutical University, Toyama, Japan.
SOURCE: Phytochemistry, Sept 1998. Vol. 49, No. 2. p. 497-505 Publisher: Oxford : Elsevier Science Ltd. CODEN: PYTCAS; ISSN: 0031-9422
NOTE: Includes references
PUB. COUNTRY: England; United Kingdom
DOCUMENT TYPE: Article
FILE SEGMENT: Non-U.S. Imprint other than FAO
LANGUAGE: English

AB 2-Hydroxyisoflavanone dehydratase, which catalyzes the final step of the formation of the isoflavonoid skeleton, was purified and characterized from yeast extract-elicited cell suspension cultures of Pueraria lobata. 2-Hydroxyisoflavanone, the substrate of the dehydratase, is the product of ***2*** - ***hydroxyisoflavanone*** ***synthase*** , as cytochrome P-450 which catalyzes the hydroxylation step associated with aryl migration of flavanone. The dehydratase was purified to apparent homogeneity for the first time by a seven-step purification procedure. It is a single polypeptide with a molecular weight of 38 kDa, and has an isoelectric point at pH 5.1 and a pH optimum at 6.8. It required no co-factor, and the apparent Michaelis constant for 2,7,4'-trihydroxyisoflavanone was 7.0 mM.

L2 ANSWER 13 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1995:694130 CAPLUS
DOCUMENT NUMBER: 124:5013
TITLE: Changes of secondary metabolism by elicitor treatment

in Pueraria lobata cell cultures

AUTHOR(S): Sankawa, Ushio; Hakamatsuka, Takashi; Shinkai, Kenji; Yoshida, Makoto; Park, Hyung-Hwan; Ebizuka, Yutaka

CORPORATE SOURCE: Faculty Pharmaceutical Sciences, University Tokyo, Tokyo, 113, Japan

SOURCE: Current Plant Science and Biotechnology in Agriculture (1995), 22(Current Issues in Plant Molecular and Cellular Biology), 595-604
CODEN: CPBAE2; ISSN: 0924-1949

PUBLISHER: Kluwer

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The cell cultures of Pueraria lobata contain isoflavone O- and C-malonylglucosides (IMG) as the main constituents. Upon treatment of P. lobata cell cultures with an elicitor yeast ext. (YE) induced the prodn. of three dimeric isoflavones, kudzuisoflavone A, B and C, which were probably formed by non-specific oxidn. of daizein with peroxidase. In contrast a biotic elicitor CuCl2 induced hypersensitive response in the cultured cells and nine isoflavonoids including a phytoalexin tuberosin and the three dimeric daizeins were produced. Treatment of the cell cultures with YE caused rapid and transient decrease of IMG within 4 h. IMG then reaccumulated and its level reached to three times higher than that of control after 100 h. CuCl2 treatment caused rapid disappearance of IMG and no reaccumulation was obsd., however enzymes and mRNAs relating to the biosynthesis of isoflavonoids in CuCl2 treated cells are higher or equal to the levels of YE treated cells. 14C-Labeled IMG expt. proved that rapid and transient decrease of IMG resulted in the deposition of isoflavones to insol. lignocellulose fraction in cell wall, which may be a rapid defense mechanism of plant resistance to outer stress.

L2 ANSWER 14 OF 14 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. (2004) on STN

ACCESSION NUMBER: 2004:22017 AGRICOLA

DOCUMENT NUMBER: IND43626355

TITLE: Isoflavonoid biosynthesis and accumulation in developing soybean seeds.

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AB Isoflavonoids are biologically active natural products that accumulate in soybean seeds during development. The amount of isoflavonoids present in soybean seed is variable, depending on genetic and environmental factors that are not fully understood. Experiments were conducted to determine whether isoflavonoids are synthesized within seed tissues during development, or made in other plant organs and transported to the seeds where they accumulate. An analysis of isoflavonoids by HPLC detected the compounds in all organs of soybean plant, but the amount of isoflavonoids present varied depending on the tissue and developmental stage. The greatest concentrations were found in mature seeds and leaves. The

2 - ***hydroxyisoflavanone*** ***synthase*** genes IFS1 and IFS2 were studied to determine their pattern of expression in different tissues and developmental stages. The highest level of expression of IFS1 was observed in the root and seed coat, while IFS2 was most highly expressed in embryos and pods, and in elicitor-treated or pathogen-challenged tissues. Incorporation of radiolabel into isoflavonoids was observed when developing embryos and other plant organs were fed with [14C]phenylalanine. Embryos excised from developing soybean seeds also accumulated isoflavonoids from a synthetic medium. A maternal effect on seed isoflavonoid content was noted in reciprocal crosses between soybean cultivars that differ in seed isoflavonoids. From these results, we propose that developing soybean embryos have an ability to synthesize isoflavonoids de novo, but that transport from maternal tissues may in part contribute to the accumulation of these natural products in the seed.

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